# ANHYDROVINBLASTINE FOR THE TREATMENT OF CANCER

## FIELD OF THE INVENTION

The present invention pertains to the field of cancer therapies and in particular to the use of the vinca alkaloid anhydrovinblastine as an anti-tumour agent.

## BACKGROUND OF THE INVENTION

Control of tumorous growth has been achieved to a certain degree using oncolytic vinca alkaloids as anti-tumour agents alone or in combination with other antineoplastic drugs in cancer chemotherapy for more than 20 years. Approximately 30 alkaloids with a wide range of pharmacological activities have been extracted from the Vinca rosea (*Catharanthus roseus*), commonly known as the periwinkle plant. Of these, only vinleurosine, vinrosidine, vinblastine and vincristine possess significant anti-tumour activity. In particular, vinblastine and vincristine have been used widely as single agents and in combination with outer antineoplastic drugs-in cancer chemotherapy. In addition to the naturally occurring alkaloids, some vinca alkaloid analogues have been synthesized by functional transformation or by semisynthetic processes (R.J. Cersosimo, *et al.*, Pharmacotherapy 3:359-274, 1983; P. Mangency, *et al.*, Org. Chem. 44:3765-3768, 1979; R. Maral, *et al.*, Cancer Lett. 22:49-54, 1984).

Chemically, these vinca alkaloids have an asymmetric dimeric structure composed of 2 nuclei linked by a carbon-carbon bond; a dihydroindole nucleus (vindoline), which is the major alkaloid contained in the periwinkle, and the indole nucleus catharanthine (Figure 1). The structural difference between vincristine and vinblastine exists at the R1 position while vinblastine and vindesine differ with regard to the R2 and R3 substituents.

The mode of action of the antineoplastic vinca alkaloids has yet to be completely understood. However, it has been established that the anti-tumour activity is directly related to the high binding affinity of these compounds for tubulin, the basic protein

subunit of microtubules (R.A. Bender and B. Chabner, In: Chabner (ed) Pharmacol. Princ. of Cancer Treat., Saunders, Phil, PA, p. 256, 1982; W.A. Creasey, In: Hahn (ed) Antibiotica, Vol. 2, Springer, Berlin, p. 414, 1979). The consensus is that these agents arrest cell mitosis at metaphase by preventing tubulin polymerization to form microtubules and by inducing depolymerization (R.J. Owellen and C.A. Hartke, Cancer Res., 36:1499-1504, 1976; R.H. Himes and R.N. Kersey, Cancer Res., 36:3798-3806, 1976; R.S. Camplejohn, Cell Tissue Kinet. 13:327-332, 1980). As such, the vinca alkaloids are cell cycle-specific anti-mitotic agents, or spindle poisons. The binding affinity of the vinca alkaloids to tubulin correlates poorly with the relative ability of vincristine, vinblastine and vindesine to inhibit cell growth (R.S. Camplejohn, supra; P.J. Ferguson and C.E. Cass, Cancer Res., 45:5480-5488, 1985). The major difference in anti-tumour activity between these drugs appears, therefore, to relate to their retention in tumour tissue (P. Ferguson, supra; J.K. Horton et al., Biochem. Pharmacol. 37:3995-4000, 1988). In a similar vein, the different toxicity profiles of the vinca alkaloids seems related to tissue uptake and retention properties rather than to inherent tubulin binding affinity. For example, studies have demonstrated that vincristine is more potent than vinblastine or vindesine in blocking fast axoplasmic transport in nerve cells (S. Ochs and R. Worth, Proc. Am. Assoc. Cancer Res., 16:70, 1975; S.Y. Chan et al., J. Neurobiol. 11:251-264, 1980). In addition, it is taken up into nerves 4 times faster than the other drugs (Z. Iqbal and S. Ochs, J. Neurobiol., 11:251-264, 1980) and exhibits an extended terminal elimination phase of plasma clearance, suggesting vincristine exposure is more prolonged than the other vinca alkaloids (R.L. Nelson et al., Cancer Treat. Rev., 7:17-24, 1980).

The *in vitro* and *in vivo* differences observed between the vinca alkaloids are striking, given the subtle chemical alterations displayed by the various agents relative to their large, complex molecular structure. For example, vincristine is very effective in treating human rhabdosarcomas transplanted in nude mice whereas vinblastine is not active in this system (N. Bruchovsky et al., Cancer Res. 25:1232-1238, 1965). This difference is obtained simply as a result of the substitution of an aldehyde group for a methyl group at the R1 position. Further, this chemical substitution leads to a shift in the toxicology profile such that peripheral neuropathy (in the absence of hematological toxicity) is dose

limiting in humans for vincristine whereas anemia and leucopenia are typically dose limiting for vinblastine (W.P. Brads, Proc. Int. Vincaalkaloid Symposium, 95-123, 1980; S.S. Legha, Med. Toxicol., 1:421-427, 1986). A particularly interesting therapeutic profile has been observed for a new semisynthetic vinca alkaloid named Navelbine™ (vinorelbine, 5'-noranhydroblastine). This compound is less potent than vinblastine and vincristine against murine P388 and L1210 leukaemia but is active against cells derived from human lung cancer whereas the other vinca alkaloids are inactive (S. Cros, *et al.*, Seminars in Oncology, 16:15-20, 1989). As well, clinical trials on Navelbine™ support its utility in treating non-small cell lung cancer (A. Depierre *et al.*, Am. J. Clin. Oncol., 14:155- 119, 1991; A. Yokoyama *et al.*, Am. Soc. Clin. Oncol., 11:957, 1992). The toxicity profile of this agent appears similar to that of vinblastine, in that hematological toxicities and not neurological side effects are dose limiting.

Vincristine has proved particularly useful as an intravenously administered oncolytic agent in combination with other oncolytic agents for the treatment of various cancers including central- nervous-system leukaemia, Hodgkin's disease, lymphosarcoma, reticulum-cell sarcoma, rhabdomyosarcoma, neuroblastoma, and Wilma tumour. It is for intravenous (IV) use only and the intrathecal administration is uniformly fatal. Following single weekly doses, the most common adverse reaction is hair loss; the most troublesome are neuromuscular in origin. When single weekly doses of the drug are employed, the adverse reactions of leukopenia, neuritic pain, constipation, and difficulty in walking can occur. Other adverse reactions that have been reported are abdominal cramps, ataxia, foot drop, weight loss, optic atrophy with blindness, transient cortical blindness, fever, cranial nerve manifestations, parehesia and numbness of the digits, polyuria, dysuria, oral ulceration, headache, vomiting, diarrhoea, and intestinal necrosis and/or perforation.

Navelbine™ (vinorelbine tartrate) is a vinca alkaloid in which the catheranthine unit is the site of structural modification. Its anti-tumour activity is also thought to be due primarily to its ability to interfere with microtubule activity thereby inhibiting mitosis at metaphase through its interaction with tubulin. It is indicated in the treatment of advanced non-small cell lung cancer as a single agent or in combination, administered

by intravenous route only. Its side effects include phlebitia or extravasion injury as it is a moderate vasicant. Studies on adverse reactions based on use of Navelbine™ as a single agent indicate Granculocytopenia as the major dose-limiting toxicity, although it was generally reversible and not cumulative over time. Mild to moderate peripheral neuropathy manifested by pareathesia and hypesthesia are the most frequently reported neurologic toxicities, occurring in 10% of patients. Mild to moderate nausea occurs in roughly one-third of patients treated with Navelbine™ with a slightly lesser fraction experiencing constipation, vomiting, diarrhoea, anorexia, and stomatitis.

A derivative of vinblastine, 3′,4′-anhydrovinblastine (AHVB or AVLB), which differs from vinblastine in that it possesses a double bond at the 3′,4′ position of the caranthine nucleus rather than the hydrogen/hydroxyl group present in the parent structure, has been described. This compound has been shown to have decreased toxicity as compared to vincristine and to inhibit the growth of lymphomas and certain solid tumours, specifically cervical, lung, breast and colon cancer (see U.S. Patent Nos. 6,011,041 and 6,326,376).

This background information is provided for the purpose of making known information believed by the applicant to be of possible relevance to the present invention. No admission is necessarily intended, nor should be construed, that any of the preceding information constitutes prior art against the present invention.

## SUMMARY OF THE INVENTION

An object of the present invention is to provide a use of 3′,4′- anhydrovinblastine (AHVB) in the treatment of cancer, and in particular in the treatment of advanced cancer. In accordance with one aspect of the present invention, there is provided a use of a therapeutically effective amount of 3', 4'-anhydrovinblastine, or a pharmaceutically acceptable salt thereof, for the treatment of cancer in a mammal in need of such therapy, wherein said cancer is pancreatic cancer, neuroendocrine cancer or soft tissue sarcoma.

In accordance with another aspect of the present invention, there is provided a use of a therapeutically effective amount of 3', 4'-anhydrovinblastine, or a pharmaceutically

acceptable salt thereof, for the treatment of advanced cancer in a mammal in need of such therapy.

In accordance with another aspect of the present invention, there is provided a use of a therapeutically effective dose of 3', 4'-anhydrovinblastine for the treatment of cancer in a mammal in need of such therapy, wherein said therapeutically effective dose is between about 2 and about 30 mg of 3', 4'-anhydrovinblastine (AHVB)/$m^2$.

In accordance with another aspect of the present invention, there is provided a use of 3', 4'-anhydrovinblastine in the manufacture of a medicament for the treatment of pancreatic cancer, neuroendocrine cancer or soft tissue sarcoma in a mammal.

In accordance with another aspect of the present invention, there is provided a use of 3', 4'-anhydrovinblastine in the manufacture of a medicament for the treatment of advanced cancer in a mammal.

In accordance with another aspect of the present invention, there is provided a use of 3', 4'-anhydrovinblastine in the manufacture of a medicament for the treatment of cancer in a mammal, wherein said medicament is formulated for administration at a dose of between about 2 and about 30 mg of 3', 4'-anhydrovinblastine (AHVB)/$m^2$.

In accordance with another aspect of the present invention, there is provided a pharmaceutical composition comprising 3′, 4′- anhydrovinblastine (AHVB) and one or more pharmaceutically acceptable, inert or physiologically active carriers, diluents or adjuvants, said AHVB being formulated for administration to a mammal at a dose of between about 2 and about 30 mg AHVB/$m^2$.

## BRIEF DESCRIPTION OF THE DRAWINGS

**Figure 1** depicts the chemical structure of some vinca alkaloids.

**Figure 2** depicts measured AHVB serum concentration over a 0 to 72 h time frame for individual patients in a Phase I clinical trial of AHVB.

**Figure 3** depicts the clearance of AHVB for patients in a Phase I clinical trial at their respective dose level.

**Figure 4** depicts the linear increase in AUC with Dose for patients enrolled in a Phase I clinical trial of AHVB.

**Figure 5** depicts the increase in Half Life with Dose for patients enrolled in a Phase I clinical trial of AHVB.

**Figure 6** depicts the linear increase in maximum plasma concentration ($C_{max}$) with Dose for patients enrolled in a Phase I clinical trial of AHVB.

**Figure 7** depicts a Goodness of Fit Plot demonstrating the ability of a two-compartment pharmokinetic model to predict the pharmokinetic properties of AHVB.

**Figure 8** depicts AHVB concentration-time profiles from a Phase I clinical trial of AHVB. The data are presented as mean $\pm$ standard error.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for the use of AHVB in the treatment of cancer in a mammal. Phase I clinical trials in which AHVB was administered to patients in dosages between about 2.5 mg/m$^2$ and about 30 mg/m$^2$ have demonstrated that AHVB is effective in the treatment of advanced cancers, including metastatic cancers.

### *Definitions*

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

The term "variants" for purposes of 3',4'-anhydrovinblastine means any chemical structure that is a derivative of 3',4'-anhydrovinblastine achieved through conservative substitution of side groups, yet still exhibits the same or similar antineoplastic properties as 3',4'-anhydrovinblastine.

"Time-to-progression" or "progression free survival," as used herein, refers to the time from the initiation of treatment (or randomisation, as the case may be) to the time of progression, or the time of death for patients who have died in the absence of progression, irrespective of the cause.

"Progression," as used herein, refers to an increase of at least 25% in the overall sum of measurable lesions as compared to nadir (*i.e.* best response) and/or the appearance of new lesions.

"Response status," as used herein, refers to a measurement of the behaviour of a tumour(s) or lesion(s) under chemotherapy, namely any observed growth (progression of disease), stability, or shrinkage (complete or partial response).

"Advanced disease," as used herein, refers to overt disease in a patient, wherein such overt disease is not amenable to cure by local modalities of treatment, such as surgery or radiotherapy. Advanced disease may refer to a locally advanced cancer or it may refer to metastatic disease.

The term "metastatic disease," as used herein, refers to a disease that has spread from one part of the body to another.

"Relapse," as used herein, refers to the relapse of a patient with advanced disease. "Relapse time," as used herein, refers to the time from the initial appearance of a primary cancer to the appearance of advanced disease requiring chemotherapy.

As used herein, the term "aggressive cancer" refers to a rapidly growing cancer. One skilled in the art will appreciate that for some cancers, such as breast cancer or prostate cancer the term "aggressive cancer" will refer to an advanced cancer that has relapsed within approximately the earlier two-thirds of the spectrum of relapse times for a given cancer, whereas for other types of cancer, such as small cell lung carcinoma (SCLC) nearly all cases present rapidly growing cancers which are considered to be aggressive. The term can thus cover a subsection of a certain cancer type or it may encompass all of other cancer types.

As used herein, the phrase "suspected of having an aggressive cancer," refers to a situation wherein a patient has had a tumour or lesion, which tumour or lesion had features correlated with the development of advanced disease, for example, markers predictive of aggressive disease. In a specific example, an indication of aggressive breast cancer is a tumour that is estrogen-receptor negative (ER–). Alternatively, the tumour may be ER positive, but the patient may exhibit other markers predictive of aggressive disease, such as node positivity. In these situations adjuvant therapies may be applied.

The term "adjuvant therapy," as used herein, refers to a treatment that is added to increase the effectiveness of a primary treatment. In cancer, adjuvant therapy usually refers to chemotherapy, hormonal therapy or radiation therapy after surgery (primary therapy) to increase the likelihood of killing all cancer cells.

The term "neoadjuvant therapy," as used herein, refers to a treatment given before the primary treatment. Examples of neoadjuvant therapy include chemotherapy, radiation therapy, and hormone therapy.

The term "hormone therapy," as used herein, refers to a treatment in which hormones or anti-hormone drugs are administered to a patient in order to slow or stop the growth of certain cancers (such as prostate and breast cancer) by blocking the body's natural hormones.

The term "hormone-resistant cancer," as used herein, refers to a cancer that does not respond to hormone therapy, whereas the term "hormone-responsive cancer" refers to a cancer that does respond to hormone therapy.

Examples of cancers which may be may be treated, stabilized, or prevented in accordance with the present invention include, but are not limited to leukaemia, carcinomas, adenocarcinomas, melanomas and sarcomas. Carcinomas, adenocarcinomas and sarcomas are also frequently referred to as "solid tumours." Examples of commonly occurring solid tumours include, but are not limited to, cancer of the brain, breast, cervix, colon, head and neck, kidney, lung, ovary, pancreas, prostate, stomach and uterus, non-small cell lung cancer and colorectal cancer.

The term “leukaemia” refers broadly to progressive, malignant diseases of the blood-forming organs. Leukaemia is typically characterized by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow but can also refer to malignant diseases of other blood cells such as erythroleukaemia, which affects immature red blood cells. Leukaemia is generally clinically classified on the basis of (1) the duration and character of the disease – acute or chronic; (2) the type of cell involved – myeloid (myelogenous), lymphoid (lymphogenous) or monocytic, and (3) the increase or non-increase in the number of abnormal cells in the blood – leukaemic or aleukaemic (subleukaemic). Leukaemia includes, for example, acute nonlymphocytic leukaemia, chronic lymphocytic leukaemia, acute granulocytic leukaemia, chronic granulocytic leukaemia, acute promyelocytic leukaemia, adult T-cell leukaemia, aleukaemic leukaemia, aleukocythemic leukaemia, basophylic leukaemia, blast cell leukaemia, bovine leukaemia, chronic myelocytic leukaemia, leukaemia cutis, embryonal leukaemia, eosinophilic leukaemia, Gross’ leukaemia, hairy-cell leukaemia, hemoblastic leukaemia, hemocytoblastic leukaemia, histiocytic leukaemia, stem cell leukaemia, acute monocytic leukaemia, leukopenic leukaemia, lymphatic leukaemia, lymphoblastic leukaemia, lymphocytic leukaemia, lymphogenous leukaemia, lymphoid leukaemia, lymphosarcoma cell leukaemia, mast cell leukaemia, megakaryocytic leukaemia, micromyeloblastic leukaemia, monocytic leukaemia, myeloblastic leukaemia, myelocytic leukaemia, myeloid granulocytic leukaemia, myelomonocytic leukaemia, Naegeli leukaemia, plasma cell leukaemia, plasmacytic leukaemia, promyelocytic leukaemia, Rieder cell leukaemia, Schilling's leukaemia, stem cell leukaemia, subleukaemic leukaemia, and undifferentiated cell leukaemia.

The term “sarcoma” generally refers to a tumour which originates in connective tissue, such as muscle, bone, cartilage or fat, is made up of a substance like embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar or homogeneous substance. Sarcomas include soft tissue sarcomas, chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, Abemethy’s sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms’ tumour sarcoma, endometrial sarcoma, stromal sarcoma,

Ewing's sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented haemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, and telangiectaltic sarcoma. In one embodiment of the present invention, AHVB is used to treat a patient with a sarcoma. In another embodiment, the sarcoma is a soft tissue sarcoma. In other embodiments, the sarcoma is a metastatic sarcoma or a metastatic sarcoma to the lungs.

The term "melanoma" is taken to mean a tumour arising from the melanocytic system of the skin and other organs. Melanomas include, for example, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, nodular melanoma, subungal melanoma, and superficial spreading melanoma.

The term "carcinoma" refers to a malignant new growth made up of epithelial cells, such growths frequently infiltrate the surrounding tissues and give rise to metastases. Exemplary carcinomas that may be treated in accordance with the present invention include, for example, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colorectal carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiermoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniform carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix

carcinoma, haematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypernephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, naspharyngeal carcinoma, oat cell carcinoma, non-small cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, and carcinoma villosum. In one embodiment of the present invention, AHVB is used to treat a patient with a carcinoma.

The term “carcinoma” also encompasses adenocarcinomas. Adenocarcinomas are carcinomas that originate in cells that make organs which have glandular (secretory) properties or that originate in cells that line hollow viscera, such as the gastrointestinal tract or bronchial epithelia. Examples include, but are not limited to, adenocarcinomas of the breast, lung, pancreas and prostate. In one embodiment of the present invention, AHVB is used to treat a patient with an adenocarcinoma. In another embodiment, the adenocarcinoma is breast cancer. In other embodiments, the adenocarcinoma is prostate cancer or pancreatic cancer.

Additional cancers encompassed by the present invention include, for example, Hodgkin’s Disease, Non-Hodgkin’s lymphoma, multiple myeloma, neuroblastoma, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, small-cell lung tumours, primary brain tumours, malignant pancreatic insulanoma, malignant carcinoid,

urinary bladder cancer, premalignant skin lesions, gliomas, testicular cancer, thyroid cancer, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, mesothelioma and medulloblastoma.

**AHVB Anti-Tumour Activity**

AHVB has shown significant cytotoxic potential against a panel of human cancer cell lines, and significant activity against the human H460 non-small cell lung carcinoma tumour xenograph in SCID/Rag-2 Mice. *In vitro* cytotoxicity assays utilizing the MTT cytotoxicity assay with a drug exposure time of 72 hours have shown that AHVB is an active cytotoxic drug with $IC_{50}$ values ranging from 20 – 24 nM against the H460 human non-small cell lung carcinoma, C-4 human cervical carcinoma, K562 human leukaemia, and the A431 human epidermoid cell lines. AHVB was approximately 10-fold less active than Navelbine™ when tested *in vitro* against the same cell lines. Surprisingly, however, when AHVB was tested *in vitro* in solid tumour efficacy experiments, it was found to be more potent than Navelbine™. Male SCID/Rag-2 mice were inoculated subcutaneous with H460 cells and after 12 days of tumour growth AHVB and Navelbine™ were delivered IV at doses of 10mg/kg and 20 mg/kg on days 1, 5 and 9. In this model, AHVB resulted in greater tumour growth inhibition and was less toxic than Navelbine™ (U.S. Patent Nos. 6,011,041 and 6,326,376). Although these results indicate that AHVB has desirable pharmacological properties for therapeutic applications, the efficacy and appropriate dosages of AHVB for the treatment of cancer in humans were not determined.

**Clinical Trials in Cancer Patients**

In order to determine the efficacy and appropriate dosages of AHVB for the treatment of cancer in humans, AHVB can be tested in Clinical Trials. As is known in the art, clinical trials progress through phases of testing, which are identified as Phases I, II, III, and IV.

Initially, AHVB was evaluated in a Phase I trial (see Examples). Typically Phase I trials are used to determine the best mode of administration (for example, by pill or by

injection), the frequency of administration, and the toxicity for the compounds. Phase I studies frequently include laboratory tests, such as blood tests and biopsies, to evaluate the effects of a compound in the body of the patient. For a Phase I trial, a small group of cancer patients are treated with specific doses of AHVB. During the trial, the dose is typically increased group by group in order to determine the maximum tolerated dose (MTD) and the dose-limiting toxicities (DLT) associated with the compound. This process determines an appropriate dose to use in a subsequent Phase II trial.

A Phase II trial can be conducted to further evaluate the effectiveness and safety of AHVB. In Phase II trials, AHVB is administered to groups of patients with either one specific type of cancer or with related cancers, using the dosage found to be effective in Phase I trials.

Phase III trials focus on determining how a compound compares to the standard, or most widely accepted, treatment. In Phase III trials, patients are randomly assigned to one of two or more "arms". In a trial with two arms, for example, one arm will receive the standard treatment (control group) and the other arm will receive AHVB treatment (investigational group).

Phase IV trials are used to further evaluate the long-term safety and effectiveness of a compound. Phase IV trials are less common than Phase I, II and III trials and will take place after AHVB has been approved for standard use.

*Eligibility of Patients for Clinical Trials*

Participant eligibility criteria can range from general (for example, age, sex, type of cancer) to specific (for example, type and number of prior treatments, tumour characteristics, blood cell counts, organ function). Eligibility criteria may also vary with trial phase. For example, in Phase I and II trials, the criteria often exclude patients who may be at risk from the investigational treatment because of abnormal organ function or other factors. In Phase II and III trials additional criteria are often included regarding disease type and stage, and number and type of prior treatments.

Phase I cancer trials usually comprise 15 to 30 participants for whom other treatment

options have not been effective. Phase II trials typically comprise up to 100 participants who have already received chemotherapy, surgery, or radiation treatment, but for whom the treatment has not been effective. Participation in Phase II trials is often restricted based on the previous treatment received. Phase III trials usually comprise hundreds to thousands of participants. This large number of participants is necessary in order to determine whether there are true differences between the effectiveness of AHVB and the standard treatment. Phase III may comprise patients ranging from those newly diagnosed with cancer to those with extensive disease in order to cover the disease continuum.

One skilled in the art will appreciate that clinical trials should be designed to be as inclusive as possible without making the study population too diverse to determine whether the treatment might be as effective on a more narrowly defined population. The more diverse the population included in the trial, the more applicable the results could be to the general population, particularly in Phase III trials. Selection of appropriate participants in each phase of clinical trial is considered to be within the ordinary skills of a worker in the art.

*Assessment of patients prior to treatment*

Prior to commencement of the study, several measures known in the art can be used to first classify the patients. Patients can first be assessed, for example, using the Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) scale. ECOG PS is a widely accepted standard for the assessment of the progression of a patient's disease as measured by functional impairment in the patient, with ECOG PS 0 indicating no functional impairment, ECOG PS 1 and 2 indicating that the patients have progressively greater functional impairment but are still ambulatory and ECOG PS 3 and 4 indicating progressive disablement and lack of mobility.

Patients' overall quality of life can be assessed, for example, using the McGill Quality of Life Questionnaire (MQOL) (Cohen *et al* (1995) *Palliative Medicine* 9: 207-219). The MQOL measures physical symptoms; physical, psychological and existential well-being; support; and overall quality of life. To assess symptoms such as nausea, mood, appetite, insomnia, mobility and fatigue the Symptom Distress Scale (SDS) developed by

McCorkle and Young ((1978) *Cancer Nursing* **1**: 373-378) can be used.

Patients can also be classified according to the type and/or stage of their disease and/or by tumour size.

*Administration of AHVB in Clinical Trials*

AHVB is typically administered to the trial participants parenterally. In one embodiment, AHVB is administered by intravenous infusion. Methods of administering drugs by intravenous infusion are known in the art. Usually intravenous infusion takes place over a certain time period, for example, over the course of 60 minutes.

A range of doses of AHVB can be tested. The studies outlined above have indicated that AHVB can be safely administered at concentrations up to ten times the dosages typically used for vinblastine. Provided with this information and effective dosages of vinblastine known in the art, a skilled practitioner could readily determine appropriate dosages of AHVB for use in clinical trials. An exemplary dose range for AHVB treatment includes dosages in the range 2.5 mg/m$^2$ to 30 mg/m$^2$.

*Pharmacokinetic monitoring*

To fulfil Phase I criteria, distribution of the AHVB is monitored, for example, by chemical analysis of samples, such as blood or urine, collected at regular intervals. For example, samples can be taken at regular intervals up until about 72 hours after the start of infusion. In one embodiment, samples are taken at 0, 0.33, 0.67, 1, 1.25, 1.5, 2, 4, 6, 8, 12, 24, 48 and 72 hours after the start of each infusion of AHVB.

If analysis is not conducted immediately, the samples can be placed on dry ice after collection and subsequently transported to a freezer to be stored at –70 °C until analysis can be conducted. Samples can be prepared for analysis using standard techniques known in the art and the amount of AHVB present can be determined, for example, by high-performance liquid chromatography (HPLC).

Pharmacokinetic data can be generated and analyzed in collaboration with an expert clinical pharmacologist and used to determine, for example, clearance, half-life and maximum plasma concentration.

*Monitoring of Patient Outcome*

The endpoint of a clinical trial is a measurable outcome that indicates the effectiveness of a compound under evaluation. The endpoint is established prior to the commencement of the trial and will vary depending on the type and phase of the clinical trial. Examples of endpoints include, for example, tumour response rate – the proportion of trial participants whose tumour was reduced in size by a specific amount, usually described as a percentage; disease-free survival – the amount of time a participant survives without cancer occurring or recurring, usually measured in months; overall survival – the amount of time a participant lives, typically measured from the beginning of the clinical trial until the time of death. For advanced and/or metastatic cancers, disease stabilization – the proportion of trial participants whose disease has stabilized, for example, whose tumour(s) has ceased to grow and/or metastasize, can be used as an endpoint. Other endpoints include toxicity and quality of life.

Tumour response rate is a typical endpoint in Phase II trials. However, even if a treatment reduces the size of a participant's tumour and lengthens the period of disease-free survival, it may not lengthen overall survival. In such a case, side effects and failure to extend overall survival might outweigh the benefit of longer disease-free survival. Alternatively, the participant's improved quality of life during the tumour-free interval might outweigh other factors. Thus, because tumour response rates are often temporary and may not translate into long-term survival benefits for the participant, response rate is a reasonable measure of a treatment's effectiveness in a Phase II trial, whereas participant survival and quality of life are typically used as endpoints in a Phase III trial.

**Pharmaceutical Compositions**

The present invention also provides pharmaceutical compositions containing AHVB in combination with one or more pharmaceutically acceptable, inert or physiologically active, carriers, diluents or adjuvants. AHVB may be freeze dried and, if desired, combined with other pharmaceutically acceptable excipients to prepare formulations for administration. If desired, the pharmaceutical compositions comprising AHVB may further comprise one or more other active ingredients, for example, other

chemotherapeutic agents useful in the treatment of cancer. These compositions are presented in a form appropriate for the administration route envisaged. In one embodiment of the invention, AHVB is formulated for parenteral administration. In another embodiment, AHVB is formulated for intravenous administration.

When formulated as an injectable composition, the pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous solution or suspension. This solution/suspension may be formulated according to known art using suitable dispersing or wetting agents and agents for dissolution or suspension of the AHVB such as those mentioned below.

Aqueous suspensions contain AHVB in admixture with suitable excipients including, for example, suspending agents, such as sodium carboxymethylcellulose, methyl cellulose, hydropropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents such as a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example, polyoxyethyene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example, hepta-decaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol for example, polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example, polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or *n*-propyl *p*-hydroxy-benzoate. Oily suspensions may be formulated by suspending the active ingredients in a vegetable oil, for example, arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin.

The sterile injectable preparation may also be sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Acceptable vehicles and solvents that may be employed include, but are not limited to, water, Ringer's solution, lactated Ringer's solution and isotonic sodium chloride solution. Other examples are, sterile, fixed oils which are conventionally employed as a solvent or suspending medium, and a variety of bland fixed oils including,

for example, synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables. When AHVB is administered parenterally in a sterile medium or vehicle, it can either be suspended or dissolved in the vehicle depending on the vehicle and concentration used. Advantageously, adjuvants such as local anaesthetics, preservatives and buffering agents can also be dissolved in the vehicle.

The pharmaceutical compositions may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions (as described above), dispersible powders or granules, emulsion hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to methods known to the art for the manufacture of pharmaceutical compositions and may contain one or more agents selected from the group of sweetening agents, flavouring agents, colouring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with suitable non-toxic pharmaceutically acceptable excipients including, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, such as corn starch, or alginic acid; binding agents, such as starch, gelatine or acacia, and lubricating agents, such as magnesium stearate, stearic acid or talc. The tablets can be uncoated, or they may be coated by known techniques in order to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate may be employed.

Pharmaceutical compositions for oral use may also be presented as hard gelatine capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatine capsules wherein the active ingredient is mixed with water or an oil medium such as peanut oil, liquid paraffin or olive oil.

Aqueous and oily suspensions containing AHVB may also contain one or more colouring agents, one or more flavouring agents or one or more sweetening agents, such as sucrose or saccharin. Oily suspensions may further contain a thickening agent, for example,

beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and/or flavouring agents may be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid.

Pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oil phase may be a vegetable oil, for example, olive oil or arachis oil, or a mineral oil, for example, liquid paraffin, or it may be a mixtures of these oils. Suitable emulsifying agents may be naturally-occurring gums, for example, gum acacia or gum tragacanth; naturally-occurring phosphatides, for example, soy bean, lecithin; or esters or partial esters derived from fatty acids and hexitol, anhydrides, for example, sorbitan monoleate, and condensation products of the said partial esters with ethylene oxide, for example, polyoxyethylene sorbitan monoleate. The emulsions may also contain sweetening and flavouring agents.

Syrups and elixirs may be formulated with sweetening agents, for example, glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, and/or flavouring and colouring agents.

AHVB may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include, for example, cocoa butter and polyethylene glycols.

Other pharmaceutical compositions and methods of preparing pharmaceutical compositions are known in the art and are described, for example, in "*Remington: The Science and Practice of Pharmacy,*" Gennaro, A., Lippincott, Williams & Wilkins, Philadelphia, PA (2000) (formerly "*Remingtons Pharmaceutical Sciences*").

The present invention also provides for formulation of AHVB into specific dosage units. Suitable dosages of AHVB determined from clinical trials are between about 2 mg/m$^2$ and about 30 mg/m$^2$. Thus, pharmaceutical compositions formulated for administration at a dose of between about 2 and about 30 mg AHVB/m$^2$ are contemplated by the present

invention. The pharmaceutical compositions may be provided as single-dose units or as multi-dose units. In one embodiment, single dose units formulated for administration at a dose of between about 5 and about 25 mg AHVB/m$^2$ are provided. In another embodiment, single dose units formulated for administration at a dose of between about 10 and about 21 mg AHVB/m$^2$ are provided.

**Administration of AHVB**

AHVB may be administered in a number of ways depending upon whether local or systemic treatment of the organism is desired. Administration may be pulmonary, *e.g.* by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal; intranasal; epidermal or transdermal; oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion, or intracranial, *e.g.* intrathecal or intraventricular, administration. For parenteral injection, AHVB or pharmaceutical compositions comprising AHVB are used in the form of a sterile solution containing other solutes, for example, enough saline or glucose to make the solution isotonic as described above. In one embodiment of the present invention, AHVB is administered by parenteral infusion. In another embodiment, AHVB is administered through intravenous (IV) infusion.

AHVB may be administered topically in a lotion or cream, for example, for application to the skin in order to treat a melanoma. Alternatively, AHVB may be administered orally in dosage forms such as tablets, capsules and the like as described above. In all cases, the proportion of active ingredients in any solid and liquid composition will be at least sufficient to impart the desired activity to the individual being treated upon oral administration. For administration by inhalation or insufflation, AHVB or pharmaceutical compositions comprising AHVB can be formulated into an aqueous or partially aqueous solution, which can then be utilized in the form of an aerosol.

The dose of AHVB to be administered, whether a single dose, multiple dose, or a daily dose, will vary and a dose regimen is designed based on such factors as the potency of the compound, the particular compositions employed, route of administration, size of the patient and the nature and severity of the patient's condition, amongst others. The dosage

to be administered is not subject to defined limits, but it will usually be an effective amount. It will also usually be the equivalent, on a molar basis, of the pharmacologically active free form produced from a dosage formulation upon the metabolic release of the active free drug to achieve its desired pharmacological and physiological effects.

Dosage requirements can be determined by standard clinical techniques, known to a worker skilled in the art. Typically, treatment is initiated with small dosages less than the optimum dose of AHVB. Thereafter the dosage is increased until the optimum effect under the circumstances is reached. In general, AHVB or pharmaceutical compositions comprising AHVB are administered at a concentration that will generally afford effective results while minimizing harmful or deleterious side effects. Administration can be either as a single unit dose or, if desired, the dosage can be divided into convenient sub-units that are administered at suitable times throughout the day. An oncologist skilled in the art of cancer treatment will be able to ascertain, without undue experimentation, appropriate protocols for effective administration of the compounds of this present invention by referring to the earlier studies of vinblastine and its derivatives.

In one embodiment of the present invention, AHVB is administered at a dose between about 2 $mg/m^2$ and about 30 $mg/m^2$. In another embodiment, AHVB is administered at a dose between about 2.5 $mg/m^2$ and about 25 $mg/m^2$. In other embodiments, AHVB is administered at a dose between about 5 $mg/m^2$ and about 25 $mg/m^2$, between about 10 $mg/m^2$ and about 25 $mg/m^2$, between about 10 $mg/m^2$ and about 21 $mg/m^2$ and between about 16.5 $mg/m^2$ and about 21 $mg/m^2$.

When AHVB is administered by intravenous infusion, the dose of AHVB is typically administered to the patient over a time period of between about 30 minutes and about 90 minutes. In one embodiment of the present invention, AHVB is administered by intravenous infusion over a time period of about 60 minutes.

Treatment regimens are typically designed such that the AHVB is administered to the patient in cycles. Treatment with AHVB in accordance with the present invention may be part of a treatment regimen that involves one cycle of administration or the regimen may involve more than one cycle. Generally, the treatment regimen involves between

about 2 and about 10 cycles. In one embodiment of the present invention, the treatment regimen involves between about 4 and about 8 cycles. In another embodiment, the treatment regimen involves about 6 cycles. Typically, a cycle is between about 1 and about 4 weeks. In one embodiment, the cycle is about 3 weeks.

The present invention also contemplates the use of AHVB in treatment regimens in conjunction with one or more chemotherapeutic agents ("combination therapies"). In such regimens, AHVB can be administered to the patient prior to, or after, administration of the one or more other chemotherapeutic agents, or it can be administered concomitantly.

When AHVB is administered prior to the one or more other chemotherapeutic agents, the length of time between administration of the AHVB and the other compound(s) will depend on the mode of administration and the size of the patient. Generally, AHVB will be administered to the patient for between about 30 minutes and about 90 minutes prior to administration of the other chemotherapeutic agent(s). Alternatively, AHVB and the other chemotherapeutic agent(s) are administered concurrently, in which case administration of the chemotherapeutic(s) may be initiated at the same time as, or at a suitable time after, initiation of administration of AHVB. Generally, administration of the other chemotherapeutic(s) is initiated about 30 minutes to about 90 minutes after administration of AHVB was initiated.

A wide range of cancer chemotherapeutic agents is known in the art. Suitable agents for use with AHVB in combination therapies include those chemotherapeutic agents which are specific for the treatment of a particular type of cancer as well as those which may be applicable to a range of cancers, for example doxorubicin, mitoxantrone, irinotecan (CPT-11). Examples of chemotherapeutic agents suitable for the treatment of breast cancer include, but are not limited to, cyclophosphamide, ifosfamide, cisplatin, carboplatin, 5-fluorouracil (5-FU), taxanes such as paclitaxel and docetaxel and various anthracyclines, such as doxorubicin and epi-doxorubicin (also known as epirubicin). Combination therapies using standard cancer chemotherapeutics may also be used in conjunction with AHVB and are also well known in the art, for example, the combination

of epirubicin with paclitaxel or docetaxel, or the combination of doxorubicin or epirubicin with cyclophosphamide, which are used for breast cancer treatments. Polychemotherapeutic regimens are also useful and may consist, for example, of doxorubicin/cyclophosphamide/5-fluorouracil or cyclophosphamide/epirubicin/5-fluorouracil. Cyclophosphamide, mitoxantrone and estramustine are known to be suitable for the treatment of prostate cancer. Cyclophosphamide doxorubicin and etoposide are used in the treatment of small cell lung cancer, as are combinations of etoposide with either cisplatin or carboplatin. In the treatment of stomach or oesophageal cancer, combinations of doxorubicin or epirubicin with cisplatin and 5-fluorouracil are useful. For colorectal cancer, CPT-11 alone or in combination with 5-fluorouracil-based drugs, or oxaliplatin in combination with 5-fluorouracil-based drugs can be used. Other examples include the combination of cyclophosphamide, doxorubicin and prednisone in the treatment of non-Hodgkin's lymphoma; the combination of doxorubicin, bleomycin, vinblastine and DTIC in the treatment of Hodgkin's disease and the combination of cisplatin or carboplatin with any one or a combination of gemcitabine, paclitaxel, docetaxel, vinorelbine or etoposide in the treatment of non-small cell lung cancer.

**Therapeutic Uses**

In clinical trials, AHVB was shown to be active in stabilising cancer in humans, in particular advanced and metastatic cancers. Phase I clinical trials in which AHVB was administered to patients as a 60 minute intravenous infusion once every 3 weeks for up to 6 courses at a dose of 10 mg/m$^2$ demonstrated stable disease in one patient with metastatic sarcoma to the lungs. At dosage levels of 21 and 25 mg/m$^2$, stable disease was shown in three patients with metastatic NSCLC. These results demonstrate that AHVB has a significant effect in humans in the treatment of cancer.

In accordance with the present invention, AHVB may be used as part of a neo-adjuvant therapy (to primary therapy), as part of an adjuvant therapy regimen, and/or for the treatment of locally advanced or metastatic disease. AHVB may also be used to treat refractory and/or advanced tumours. It is further contemplated that AHVB can be used to treat patients that have undergone one or more prior courses of chemotherapy.

Primary therapy is understood to encompass a first line of treatment upon the initial diagnosis of cancer in a patient. Exemplary primary therapies may involve surgery, a wide range of chemotherapies and radiotherapy.

Adjuvant therapy is understood to encompass any therapy, following a primary therapy such as surgery that is administered to patients at risk of relapsing. Adjuvant systemic therapy is begun soon after primary therapy to delay recurrence, prolong survival or cure a patient. It is contemplated that AHVB can be used alone or in combination with one or more other chemotherapeutic agents as part of an adjuvant therapy.

In the application of cancer therapies a patient's response status is monitored to determine the effectiveness of the therapy. Arising out such monitoring may be the observation of relapse in a patient, which may refer to the relapse of a patient with advanced disease.

In one embodiment of the present invention, AHVB is used alone or in combination with one or more other chemotherapeutics in the treatment of advanced disease in a cancer patient. In another embodiment, the advanced disease is a solid tumour. In another embodiment, the advanced disease is a metastatic disease. In other embodiment, the advanced disease is metastatic sarcoma. In other embodiments, the advanced disease is metastatic neuroendocrine cancer or metastatic non-small cell lung carcinoma.

A "refractory" cancer or tumour refers to a cancer or tumour that has not responded to treatment. In accordance with the present invention, AHVB alone or in combination with one or more other chemotherapeutics can be used to treat a refractory cancer. In one embodiment, AHVB is used to treat refractory non-small cell lung carcinoma. In another embodiment, AHVB is used to treat refractory colorectal carcinoma. In another embodiment, AHVB is used to treat refractory soft tissue sarcoma. In another embodiment, AHVB is used to treat refractory pancreatic cancer. In another embodiment, AHVB is used to treat refractory breast cancer.

In yet another embodiment of the present invention, AHVB alone or in combination with one or more other chemotherapeutics can be used to treat a patient suspected of having an aggressive cancer, such as a patient has a hormone-resistant cancer. In another

embodiment, AHVB alone or in combination with one or more other chemotherapeutics is used to treat breast cancer patients with estrogen-receptor negative (ER–) tumours.

## EXAMPLES

### EXAMPLE 1: Phase I Clinical Trials

*Summary*

*Purpose:* A phase I trial was conducted to determine the maximum tolerated dose (MTD), dose-limiting toxicities (DLT) and pharmacokinetics of AHVB given as a 1-h intravenous infusion once every 3 weeks in patients with advanced refractory solid tumours.

*Patients and methods:* Entered into the study were 24 patients with normal bone marrow, hepatic and renal function, and of these 21 were evaluable. There were 12 males and 12 females with a median age of 60 years (range 27 –75 years). Diagnoses were non-small-cell lung cancer (NSCLC) (11), colorectal cancer (5), soft tissue sarcoma (4) and miscellaneous (including pancreatic cancer, breast cancer and metastatic neuroendocrine cancer and renal cell carcinoma: 4). Patients had had a median of three prior chemotherapy regimens (range one to six). A total of 51 courses were administered at doses of 2.5, 5, 10, 16.5, 21, 25 and 30 mg/m$^2$ in one, three, one, three, six, six and one patient, respectively.

*Results:* Grade 2 infusional hypertension, anaemia, and dizziness were noted at 16.5 mg/m$^2$. At 25 mg/m$^2$ two of six evaluable patients had DLT. DLT was grade 4 constipation, neutropenia and grade 3 nausea/vomiting. At 21 mg/m$^2$ one of six evaluable patients had DLT (grade 3 nausea/vomiting). This dose was the MTD. Stable disease was noted in one patient with metastatic sarcoma to the lungs and in three patients with metastatic NSCLC. The pharmacokinetics of AHVB were linear and well characterized by a two-compartment model, with a mean clearance of 26.4 l/h per m$^2$ and median terminal half-life of 18 h.

*Patients and methods*

*Eligibility*

Patients with histologically documented, advanced solid malignancies refractory to conventional therapy, or for whom no effective therapy existed were entered. Inclusion criteria included: age ≥18 years, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 –2 and a life expectancy of ≥12 weeks; no chemotherapy or radiotherapy in the 4 weeks prior to entering the study (6 weeks for nitrosoureas, mitomycin C or radiotherapy to more than 20% of bone marrow); ANC ≥1500/μl, platelet count ≥100,000/μl, total serum bilirubin ≤1.3 mg/dl, AST and ALT ≤2.5 times the upper limit of the institutional normal, and serum creatinine ≤1.5 mg/dl or creatinine clearance ≥60 ml/min; no uncontrolled brain metastases or general medical problems, and no neurotoxicity from previous therapy greater than National Cancer Institute Common Toxicity Criteria (NCI CTC) grade 2. All patients gave written informed consent according to federal and institutional guidelines before treatment.

*Dosage and drug administration*

Prescient NeuroPharma of Vancouver, Canada, supplied the AHVB in single-dose vials. The AHVB was infused intravenously over 1 h in 250 ml 5%dextrose in water within 8 h of dilution. The drug was given every 3 weeks without premedication. The starting dose was 2.5 mg/m$^2$ (one-seventh of the MTD in the rat, the more sensitive of the species studied). Dose escalation was based on the continual reassessment method (CRM) modified by Faries [*J. Biopharm Stat* 4:147 (1994)] according to pre-established dose levels [O'Quigley *et al., Biometrics*, 46:33 (1990)]. Toxicity was graded according to the NCI CTC version 2.0. One patient was treated at each non-toxic or minimally toxic dose level. If toxicity of grade 1 or more was seen, the cohort was expanded to three patients unless dose-limiting toxicity (DLT) was seen. DLT was defined as: grade 4 neutropenia for more than 7 days, febrile neutropenia or grade 4 thrombocytopenia, grade 3 non-hematologic toxicity except for controllable nausea/vomiting or diarrhea. DLT was defined on the first cycle only for dose escalation to the next level. Cumulative toxicity was noted for all cycles. There was no intrapatient escalation. Patients who were not

eligible and/or not evaluable for toxicity during the first cycle were replaced. Weekly evaluations were performed. No dose escalation was made before the 4-week evaluation of the first cycle of the last patient included at each dose level. The MTD (the highest dose at which not more than one in six patients showed DLT) was determined in the standard way [Simon *et al., J. Natl Cancer Institute,* 89:1138 (1997)].

*Pretreatment and follow-up studies*

History, physical examination and routine laboratory evaluation were performed before treatment and weekly during treatment. Laboratory evaluation included CBC, chemistry, and urinalysis. Pretreatment studies also included a chest radiograph and radiologic studies to evaluate all sites of disease. The imaging studies were repeated every other course. Patients were able to continue treatment in the absence of progressive disease, defined as a 25% increase in the size of at least one bidimensionally or unidimensionally measurable lesion over baseline, or the appearance of a new lesion.

*Plasma sampling and assay*

AHVB was administered intravenously over 1 h. Blood (7 ml) for pharmacokinetics (PK) was collected before, at 0.25, 0.5 and 1 h during infusion, and at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 48 and 72 h following the end of infusion. Samples were immediately centrifuged, and the plasma harvested and stored frozen at –20°C until assayed. Samples were assayed by a validated liquid chromatography/mass spectrometry (LC/MS) assay. The instrumental set-up included a Hewlett Packard Series II 1090 HPLC and Insersil column ODS 2, a Fisons VG Quattro 1B mass spectrometer no.5763 in atmospheric pressure chemical ionization (APCI) mode, and a Pierce "reacti-Therm" module evaporator. The assay was linear over the range 10 –2000 ng/ml, with a lower limit of quantitation of 10 ng/ml and a minimum RSQ of 0.99987.The interday %RSD of quality control samples ranged from 2.1% to 10.4%. Vincristine was used as the internal standard and AHVB had a retention time of approximately 10 min. All samples were assayed in duplicate and in random order.

*Pharmacodynamics*

The relationships between AHVB PK and both gastrointestinal and hematologic toxicities were evaluated. Nausea/vomiting and constipation were combined into one category for analysis and hematologic toxicities were divided into either leukopenia/neutropenia or anemia/thrombocytopenia. Only cycle-1 toxicities were evaluated, with graded toxicities considered as categorical variables and AUC, $C_{max}$ and dose considered as continuous variables. Recursive partitioning (Tree-based modeling, SYSTAT 10) was used to identify significant breakpoints between the prevalence of toxicity and PK parameters. Nonparametric procedures (Kruskal-Wallis or Mann-Whitney) were utilized to test for statistically significant differences between groups.

***Results***

*General*

A total of 24 patients were treated with 51 courses of AHVB through seven dose levels. Three patients were not evaluable (one brain metastases, one early disease-related death, one withdrawal after an acute drug reaction). All patients had received prior chemotherapy, including 13 who had previously received a taxane, 3 who had received a vinca and 13 who had received radiation and chemotherapy. The median number of prior chemotherapy regimens was three (range one to six courses). The starting dose was 2.5 mg/m$^2$. Three patients were enrolled at 5 mg/m$^2$ because the first had grade 2 toxicity (in the form of grade 2 anorexia, hyperamylasemia and increased serum creatinine). Five patients were enrolled at 16.5 mg/m$^2$ (two unevaluable). Grade 2 toxicities including infusional hypertension, anemia and dizziness were noted. Six patients were entered at 25 mg/m$^2$. DLT, requiring hospitalization, was noted in two patients and included grade 4 constipation and grade 3 nausea/vomiting. This dose level exceeded the MTD. Since no severe toxicities were seen at 16.5 mg/m$^2$ and the increment to 25 mg/m$^2$ represented a 50% increase, six evaluable patients were entered at 21 mg/m$^2$; one of the six had DLT (grade 3 nausea/vomiting) and grade 2 constipation, requiring brief hospitalisation, laxatives and administration of intravenous fluids. Seven patients were enrolled at the 21 mg/m$^2$ dose level, but only 6 were evaluable for toxicity due to one patient developing Grade 2 hypertension, headache, nausea and vomiting at the start of the infusion, which

recurred on rechallenge. This patient received only 15 ml of the drug solution and, therefore, was not evaluable to assess toxicity. One patient enrolled at 30 mg/m$^2$ before it was recognized that the grade 3 nausea/vomiting at 25 mg/m$^2$ was drug related had grade 3 leukopenia and neutropenia and grade 1 anaemia.

*Hematologic toxicity*

Four patients treated at dose levels 21 and 25 mg/m$^2$ showed grade 3 (three patients) and grade 4 (one patient) neutropenia. The duration of the grade 4 neutropenia was 2 days; this patient also had grade 3 anaemia. The onset of neutropenia was typically delayed; the median time to nadir was 14 days (range 2 –22 days). There were no treatment delays secondary to neutropenia. The nadir neutrophil platelet counts are given in Table 3.

*Nonhematologic toxicity*

The most common non-hematologic effects of AHVB were dose-limiting nausea/vomiting and constipation (Table 4). Of the 21 evaluable patients, 13 (61%) had grade 1 –2 nausea/vomiting. At 25 mg/m$^2$, two patients had DLT. Both had grade 3 nausea/vomiting uncontrolled by outpatient antiemetics such as prochlorperazine, and one also had grade 4 constipation (paralytic ileus) and grade 4 hematologic toxicity (febrile neutropenia). At 21 mg/m$^2$, one patient had DLT (nausea/vomiting).

*Cumulative toxicity*

Two patients received more than two cycles of AHVB. One, who received 14 cycles at 25 mg/m$^2$ exhibited transient grade 3 leukopenia and neutropenia with cycles 7 and 10, and also grade 3 nausea/vomiting and dehydration with cycle 8.

*Anti-tumour activity*

Four patients (one sarcoma at 10 mg/m$^2$, three NSCLC at 21 and 25 mg/m$^2$) had stable disease when evaluated after two courses of AHVB. One (NSCLC at 25 mg/m$^2$) had stable disease through 14 cycles and showed no evidence of progression. The median time to progression was 102 days (range 87 – 639+ days).

*Pharmacokinetics*

Plasma concentration data for AHVB were available in 20 patients. $C_{max}$ ranged from 28.2 ng/ml at 2.5 mg/m$^2$ to 571 ng/ml at the 30 mg/m$^2$. The data fitted a two-compartment PK model, with a median (range) $r^2$ of 0.89 (0.78 – 0.96). The median $t_{1/2\alpha}$ and $t_{1/2\beta}$ were 0.17 and 17.6 h (mean values were: $t_{1/2\alpha}$ 0.19 h; $t_{1/2\beta}$ 20.8 h). The mean clearance (CV%) and volume of distribution (CV%) of AHVB were 26.4 l/h per m$^2$ (49%) and 45 l/m$^2$ (34%), respectively. Figure 2 shows the time-course plots of individual patient AHVB serum concentrations, fit by a 2-compartment linear pharmacokinetic model. Figure 3 shows the clearance of AHVB for each patient at their respective dose level. A minority of subjects demonstrated evidence of a third elimination phase, which could not be characterized. AHVB exhibited linear PK over the range of doses studied, with no significant change in clearance with increasing dose (P >0.05, Kruskal-Wallis) (Figure 8). Similarly, the AUC increased linearly with dose ($r^2$ 0.82, P <0.05 by linear regression) (Figure 4). A similar linear relationship was observed between $C_{max}$ and dose (Figure 5). A goodness of fit plot is shown in Figure 7 demonstrating the model predicted versus measured AHVB concentrations.

*Pharmacodynamics*

The relationships between cycle-1 toxicities and AUC, $C_{max}$ and dose were evaluated. $C_{max}$ was a better predictor of AHVB toxicity than either dose or AUC. $C_{max}$ was associated with both the occurrence of gastrointestinal toxicity and neutropenia (P <0.05). Recursive partitioning identified significant $C_{max}$ breakpoints of approximately 250 ng/ml for both toxicities. Four of the eight patients with AHVB $C_{max}$ >250 ng/ml exhibited significant gastrointestinal toxicity (one grade 2, two grade 3, one grade 4), in contrast to none of 12 patients with $C_{max}$ <250 ng/ml (P <0.05). Similarly, four of eight patients with a $C_{max}$ >250 ng/ml had cycle-1 neutropenia (three grade 3, one grade 4), in contrast to none of 12 patients with a $C_{max}$ <250 ng/ml (P <0.05).

*In summary:* AHVB can safely be administered every 3 weeks at a dose of 21 mg/m$^2$. The DLT is nausea/vomiting, which could be early or late. The prophylactic use of 5HT3 antagonists should be considered. The prophylactic use of laxatives should be considered

in patients on narcotics who receive AHVB. The PK of AHVB are linear at doses of 2.5 to 30 mg/m$^2$, and are well described by a two-compartment model. The human PK appear similar to those of vinblastine, which has a terminal elimination half-life of approximately 20 – 25 h [Faries, *J. Biopharm Stat* 4:147 (1994); Nelson, *Cancer Treat Rev* 7:17 (1980); Owellen, *Cancer Res* 37:2597 (1977)], and a clearance of approximately 33 l/h per m$^2$ following bolus dosing [Ratain, *et al., Clin Pharmacol Ther* 41:61 (1987)]. The data above indicate toxicity was best correlated with $C_{max}$, therefore, an alternate weekly dosing schedule could be instigated.

The embodiments of the invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

**Table 1: Patient dosage levels and associated toxicities**

| Dose Level | Dose Concentration ($mg/m^2$) | Patient(s) treated |
|---|---|---|
| 1 | 2.5 | One patient treated |
| 2 | 5.0 | One patient treated developed Grade 2 elevated amylase, elevated creatine and anorexia requiring an additional 2 patients to be added at this dose |
| 3 | 10 | One patient treated with minimal toxicity |
| 4 | 16.5 | One patient treated expired 4 days after beginning cycle due to disease process (unrelated to drug study). The patient was replaced by another patient which was found to have brain metastasis requiring radiation therapy and was replaced. The next patient showed Grade 2 toxicities (anemia, hypertension, tachycardia, diaphoresis, flushing and fatigue) requiring 2 more patients at this dose. These two patients had minimal toxicities. |
| 5 | 25 | Six patients were treated with 2 having DLTs in the form of grade 4 constipation, Grade 3 tumour pain, nausea, vomiting, anemia and Grade 4 neutropenia (1 patient). |
| 6 | 30 | One patient treated developed Grade 3 leukopenia. |
| 7* | 21 | Seven patients enrolled but only 6 were evaluable for toxicity. One patient was replaced since treatment was incomplete and non-evaluable. At this dose level, one patient exhibited Grade 3 nausea and vomiting. This does was determined to be the MTD and the study was closed. |

* Since minimal toxicity was seen at 16.5 $mg/m^2$ dose level and the increment from 16.5 to 25 $mg/m^2$ represented a 50% increase, it was elected to evaluate an intermediate dose level at 21 $mg/m^2$.

**Table 2: Anhydrovinblastine Pharmacokinetic Parameters**

| Dose /mg/m² | N | | A /ng/mL | $T_{1/2}$ al /h | AUC /ug/mL*h | B /ng/mL | $T_{1/2}$ be /h | CL /L/h/m² | $C_{max}$ /ng/mL | $V_{ss}$ /L/m² |
|---|---|---|---|---|---|---|---|---|---|---|
| 2.5 | 1 | | | | | | | | | |
| | | Value | 134.71 | 0.12 | 71.64 | 5.03 | 6.63 | 34.90 | 28.23 | 226.04 |
| 5 | 3 | | | | | | | | | |
| | | Mean | 281.82 | 0.14 | 571.75 | 8.04 | 44.36 | 10.61 | 51.37 | 534.05 |
| | | Min | 135.55 | 0.07 | 282.20 | 6.27 | 27.54 | 6.30 | 38.65 | 445.34 |
| | | Median | 178.39 | 0.17 | 639.67 | 8.11 | 42.41 | 7.82 | 53.07 | 534.71 |
| | | Max | 531.52 | 0.17 | 793.39 | 9.73 | 63.13 | 17.72 | 62.40 | 622.10 |
| | | CV% | 77.11 | 41.87 | 45.87 | 21.53 | 40.30 | 58.42 | 23.28 | 16.55 |
| 10 | 1 | | | | | | | | | |
| | | Value | 109.85 | 0.25 | 247.19 | 11.49 | 12.50 | 40.45 | 48.57 | 614.22 |
| 16.5 | 5 | | | | | | | | | |
| | | Mean | 509.47 | 0.22 | 824.58 | 22.50 | 21.35 | 25.13 | 163.03 | 486.18 |
| | | Min | 263.49 | 0.13 | 511.26 | 6.64 | 8.21 | 9.21 | 96.35 | 298.06 |
| | | Median | 478.82 | 0.20 | 541.21 | 21.87 | 21.82 | 30.49 | 171.48 | 485.95 |
| | | Max | 816.42 | 0.39 | 1791.22 | 34.16 | 33.52 | 32.27 | 231.52 | 625.73 |
| | | CV% | 41.58 | 46.33 | 66.79 | 51.82 | 47.25 | 36.55 | 30.17 | 28.34 |
| 21 | 3 | | | | | | | | | |
| | | Mean | 774.37 | 0.26 | 1094.91 | 25.92 | 22.88 | 19.87 | 257.21 | 488.51 |
| | | Min | 322.90 | 0.19 | 918.58 | 24.50 | 19.00 | 14.94 | 179.07 | 471.27 |
| | | Median | 802.43 | 0.20 | 964.03 | 24.72 | 19.15 | 21.78 | 252.10 | 486.76 |
| | | Max | 1197.78 | 0.39 | 1402.11 | 28.54 | 30.50 | 22.86 | 340.46 | 507.51 |
| | | CV% | 56.58 | 43.26 | 24.39 | 8.76 | 28.83 | 21.51 | 31.42 | 3.72 |
| 25 | 6 | | | | | | | | | |
| | | Mean | 1122.91 | 0.18 | 764.83 | 34.51 | 10.68 | 36.43 | 273.43 | 380.18 |
| | | Min | 379.79 | 0.11 | 436.85 | 20.78 | 6.73 | 20.80 | 108.52 | 227.92 |
| | | Median | 1062.6 | 0.16 | 750.85 | 35.06 | 10.54 | 33.48 | 287.71 | 270.11 |
| | | Max | 1952.78 | 0.36 | 1202.01 | 48.76 | 16.29 | 57.23 | 368.79 | 762.66 |
| | | CV% | 59.00 | 49.42 | 35.74 | 32.63 | 33.27 | 36.30 | 34.30 | 55.41 |
| 30 | 1 | | | | | | | | | |
| | | Value | 3559.02 | 0.10 | 1797.92 | 36.71 | 23.83 | 16.69 | 571.08 | 403.55 |
| All | 20 | | | | | | | | | |
| | | Mean | | 0.19 | | | 20.8 | 26.4 | | 451 |
| | | Min | | 0.07 | | | 6.63 | 6.30 | | 226 |
| | | Median | | 0.17 | | | 17.60 | 25.30 | | 479 |
| | | Max | | 0.393 | | | 63.10 | 57.30 | | 763 |
| | | CV% | | 47 | | | 68 | 49 | | 34 |

**Table 3: Hematologic toxicity of AHVB (one cycle only).** One patient had a platelet count of 98 x $10^9$/l at 30mg/$m^2$; this was the only thrombocytopenia seen.

| Dose (mg/$m^2$) | Number of Patients/Courses | ANC nadir (μl) | | Number of Patients With Neutropenia | |
|---|---|---|---|---|---|
| | | Median | Range | Grade 3 | Grade 4 |
| 2.5 | 1/1 | 4.70 | - | 0 | 0 |
| 5.0 | 3/6 | 5.97 | 4.57-8.14 | 0 | 0 |
| 10.0 | 1/4 | 5.97 | - | 0 | 0 |
| 16.5 | 3/6 | 2.44 | 2.09-3.11 | 0 | 0 |
| 21.0 | 6/10 | 2.90 | 0.97-5.68 | 1 | 0 |
| 25.0 | 6/23 | 1.03 | 0.25-4.13 | 1 | 1[a] |
| 30.0 | 1/2 | 0.96 | - | 1 | 0 |

[a]The neutropenia lasted 2 days.

**Table 4: Nonhematologic toxicity of AHVB (one cycle only).** At doses of 2.5 to 10 mg/m$^2$, five patients received 11 courses; one grade 2 nausea/vomiting was seen.

| Dose (mg/m$^2$) | No. of patients/courses | Constipation | | Nausea/Vomiting | | Tumor pain | | Neurotoxicity | Arthralgia/ myalgia | Hypertension | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Grade 1/2 | Grade 3/4 | Grade 1/2 | Grade 3/4 | Grade 1/2 | Grade 3/4 | Grade 1/2 | Grade 1/2 | Grade 1/2 | Grade 3/4 |
| 16.5 | 3/6 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 3 | 0 |
| 21 | 6/10 | 3 | 0 | 3 | 1 | 0 | 1 | 3[a] | 2 | 0 | 0 |
| 25 | 6/23 | 3 | 1 | 2 | 1 | 1 | 1 | 0 | 2 | 1 | 1 |
| 30 | 1/2 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | 0 | 0 |

[a]Peripheral neuropathy